4. INTRODUCTION

4.1 Investigational Plan

This study proposes to combine Allovectin-7 gene therapy and recombinant IL-2 (rIL-2) protein therapy in a Phase I protocol to assess safety and response in patients with advanced head and neck cancer or metastatic lung cancer. This combination approach is intended to stimulate an immune response by expressing HLA-B7 antigen within the tumor, inducing an allogeneic response and potentially restoring some degree of major histocompatibility complex (MHC) class I tumor antigen presentation, while augmenting the host immune response by expanding stimulated NK cells, helper T cells and specific cytotoxic T cells through the administration of low-dose IL-2.

5. BACKGROUND AND RATIONALE

5.1 Overview

Human tumors are immunogenic and can induce anti-tumor responses (1-3). Various approaches to immunotherapy, including interferon alpha (IFN-α), interleukin-2 (IL-2) and specific tumor vaccines, induce remissions in patients with a variety of cancers including melanoma, renal cancer and breast cancer (4-9). In these studies, complete or partial remissions have been observed in 10 to 35% of patients.

Gene therapy with gene-modified tumor cell vaccines offers the promise to improve upon these statistics. In animal models, vaccination protocols with gene-modified tumor cells have proven superior to non-modified tumor cells. Cells are transfected or transduced with genes encoding various immunomodulatory proteins including: IL-2, IL-4, IL-7, IL-12, IFN-α , TNF-α or GM-CSF. These cells do not grow when implanted into syngeneic mice, and induce protection to subsequent challenge with wild-type tumor cells. However, this protection is often not induced by preimmunization with wild-type tumor cells. Also, the effect of immunization with gene-modified cells is usually abrogated by antibody to CD8 + cells suggesting the induction of tumor-specific T cell mediated immunity.

In one experiment, G. Nabel and co-workers (University of Michigan) used the insertion of an allogeneic MHC class I antigen into murine tumor cells *in vivo* to induce an allogeneic response against the tumor (10). In the course of the allogeneic response, an immune response was also induced against tumor-associated antigens of the wild type tumor. Both transfected and non-transfected tumor cells were killed by a cytotoxic T cell response. Survival was prolonged.

This work was translated into a clinical study. Five HLA-B7 negative patients with metastatic melanoma received intratumoral injections of the HLA-B7 gene in a plasmid delivered via a cationic lipid vector. The HLA-B7 DNA, mRNA and protein were detected in injected tumors from 4/5, 4/5, and 5/5 patients, respectively, and one patient had a partial clinical remission (11).

A Phase I study of direct intratumoral injection of the HLA-B7/β2M genes formulated with cationic lipid (Allovectin-7) was then carried out by us and our co-workers in 17 patients with melanoma, 14 patients with renal cell carcinoma and 15 patients with colon cancer (12,13). All of these patients were HLA-B7 negative. Of 14 evaluable melanoma patients, seven showed regression of the injected nodules. Eleven of these patients, as per protocol, received only one dose while three received 2 injections and three received 3 injections. Treatment was well tolerated. The majority of adverse events were attributable to underlying disease or study-required drug injections and biopsy procedures. Of the adverse events considered drug related, all but one were rated mild to moderate and 23/27 were due to pain, inflammation or other

injection or biopsy complications. None of the AEs rated serious were considered related to the study drug. Two IND safety reports were filed with the FDA during the study. One was for severe pain and the development of hypotension following the injection of a groin nodule; the other was for development of a subcapsular hematoma and hypotension following the injection and biopsy of a liver nodule. The *Investigator's Brochure for Allovectin-7* contains additional details of serious adverse events.

Based on the results of this Phase I study, a Phase II study has been conducted in patients with melanoma, renal cell cancer, breast cancer, colon cancer and non-Hodgkin's lymphoma.

Nabel and co-workers have completed a second study in 10 melanoma patients and observed 2 responses, including a partial remission (14). H. Silver and co-workers (Vancouver British Columbia Cancer Agency) also conducted a study in 7 melanoma patients and observed 3 responses (15). Three of seven patients and two of the responders were HLA-B7 positive.

Overall, in melanoma, a total of 36 patients have been treated with Allovectin-7. Thirty-six percent had regression in the injected nodule of >25%, and 19% had >25% regression in the size of non-injected, distant nodules.

A draft analysis of the Phase II study in melanoma has been completed recently. Of thirty-six entered patients, 27 were evaluable. Local response was observed in 26% of injected nodules, and 3 of the 27 patients or 11% had a partial remission. Thus, the biological activity of this approach has been confirmed.

There is evidence in animal systems that several cytokines can augment the immune response to a variety of antigens, including microbial antigens and tumor antigens. Systemically administered cytokines, including IFN-α and IL-2, significantly augment the development of tumor immunity to tumor cell vaccines and induce a greater generation of CTL than tumor cells alone (16-18).

The immunomodulatory doses and schedules of both IFN-α and IL-2 have been well worked out in humans (19,20). Therefore, their use as immunomodulators for tumor vaccines has a rational scientific basis.

Furthermore, these immunomodulatory doses are relatively non-toxic. Long-term subcutaneous IL-2 administration has proven to be safe, non-toxic and to improve the overall immune responsiveness of cancer patients (21-26).

Based on the above, we have hypothesized that a combination of intratumoral gene therapy plus systemic administration of immunomodulatory cytokines should induce more effective anti-tumor immunity and clinical responses in cancer patients.

Therefore, this Phase I protocol will investigate intratumoral injection of allogeneic HLA-B7 gene plus systemic administration of a cytokine for treatment of metastatic lung cancer and advanced head and neck cancers. Specifically, this protocol will study intratumoral administration of HLA-B7 concurrent with systemic IL-2 in a phase I study. In particular, we are interested in determining if subcutaneous, long-term administration of low-dose IL-2 enhances the development of systemic anti-tumor immunity and results in regression of non-injected nodules.